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(54) Title: PREPARATION FOR THE ENHANCEMENT OF THE ANTIOXIDANT STATUS OF CELLS

(57) Abstract

The invention relates to a preparation comprising a mixture of antioxidants, wherein the mixture comprises at least (a) a mixture of at least two naturally occurring carotenoids, (b) a naturally occurring tocopherol or a derivative thereof, and (c) vitamin C, wherein the naturally occurring carotenoids are in the form of carotenoid containing particles having an average diameter of 0.01 to 100 μm.

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Preparation for the enhancement of the antioxidant status of cells

Field of the Invention

The present invention relates to a preparation for the enhancement of the antioxidant status of cells which comprises a mixture of antioxidants. More particularly, the present invention relates to the application of a mix of naturally occurring antioxidants having antioxidant properties in nutrient compositions including food products, dietary/foods, food supplements, pharmaceutical compositions and in cell, tissue, culture and fermentation media.

Background of the invention

Carotenoids work in concert within a cascade of antioxidants including vitamin E and C in the protection of cells against oxidants such as reactive oxygen species (ROS) including free radicals. Cells are exposed to ROS as a consequence of the involvement of oxygen in metabolic processes in cells. Mammals, e.g. humans, can also be exposed to oxidants as a result from e.g. pollution of air with a variety of oxidants such as ozone and oxides of nitrogen. Exposure of the skin to sunlight results in the formation of ROS in the skin.

An excess of ROS in cell culture media leads to sub-optimal growth and maintenance of cells whereas oxidative stress in the human body is thought to be involved in ageing and diseases such as cardiovascular disease, certain (epithelial) cancers and visual impairments such as cataract and age-related macular degeneration (AMD).

The involvement of oxygen in metabolic processes in living cells results in the formation of highly unstable ROS. ROS such as singlet oxygen, superoxide, hydroxyl and nitric oxide, hydrogen peroxide, hypochlorous acid and peroxynitrite, are formed continuously in living cells as a consequence of metabolic and other biochemical reactions as well as external factors. Some of these ROS, e.g. superoxide and nitric oxide, can be physiologically useful, but they can also cause damage under certain circumstances. A significant amount of ROS is generated during mitochondrial

oxidative phosphorylation. Several studies have suggested that mitochondrial DNA accumulate more oxidative DNA damage relatively to nuclear DNA.

Industrialization has resulted in pollution of air with a number of chemicals known to be hazardous to human health. These chemicals include a variety of gases such as ozone and nitrogen oxides. Major sources of nitrogen dioxide are cigarette smoke and exhaust gases of combusting engines. Oxidative stress induced by cigarette smoke can alter the levels of a form of oxidative DNA damage, 8-hydroxyguanine, and its repair activity in leucocytes. Photolytic degradation of nitrogen dioxide by sunlight (ultraviolet radiation) liberates oxygen atoms which can combine with molecular oxygen to form ozone. The oxidants ozone and nitrogen dioxide have a stimulating role in the pathogenesis of various lung diseases including chronic bronchitis, emphysema and lung cancers.

Sunlight exposure of the skin results in skin injury. The short-term effects include sunburn and tanning whereas cumulative UV exposure results in photoaging and increased risk of skin cancer. At least a portion of the adverse effects seem to be mediated by ROS which causes lipid peroxidation and cellular damage in skin tissues.

Excess production of ROS may result in pathological stress to cells and tissues. This oxidative stress can have multiple effects. It can induce defence systems, and render tissues more resistant to subsequent insult. If oxidative stress is excessive or if defence and repair responses are inadequate, such mechanisms may cause cell injury such as oxidative damage to essential proteins, lipid peroxidation, DNA strand breakage and base.

The balance between antioxidants and (pro)oxidants in living organisms is defined as the antioxidant status. This balance is dynamic and, in the human body, is tipped slightly in favour of oxidation, as evidenced by ongoing damage of DNA, lipids, carbohydrates, proteins, uric acid, etc. The mammalian body has been adapted to this slight imbalance by the presence of repair mechanisms. In addition, gradual and modest increase in oxidation induces production of endogenous antioxidants, thus providing a type of feedback-regulation mechanism. Oxidative stress, a serious imbalance favouring oxidation, is said to result from excessive

production of ROS or from weakening of the antioxidant defense system. Severe oxidative stress may be important for good health and prevention of disease. If the oxidative stress is particularly severe, it can induce the death of cells in culture.

The accumulation of advanced glycation end products (AGE's), the irreversibly formed products from non-enzymatic glycation and oxidation of proteins and lipids, occurring with ageing can trigger a series of cellular events such as cellular oxidative stress, expression of leucocyte adhesion molecules, endothelial trans-migration of monocytes and smooth muscle cell chemotaxis which are all considered as important prelesional events in the atherogenesis process.

The antioxidant status is determined by factors affecting the antioxidant system and/or by the production of ROS. Diet, food and alcohol, more than any other factors, directly affect both the antioxidant system - by supplying dietary antioxidants and cofactors of endogenous antioxidants - and production of ROS. Major components of the antioxidant system derived from the diet comprising fruit and vegetables include carotenoids, tocopherols and tocotrienols (vitamin E), ascorbate (vitamin C), polyphenols including flavonoids etc. Other nutrients are essential for normal function of endogenous systems; minerals Cu, Mn, Zn, Se and Fe are important cofactors of antioxidant enzyme systems. Cell and tissue levels of dietary antioxidants are affected directly by the amount and food composition of the diet and by supplementation.

The amount of fruit and vegetables consumed in the western world is much lower than is preferred for the protection against degenerative diseases associated with oxidative stress. One of the protecting effects of fruit and especially of vegetables is its deliverance of antioxidants such as carotenoids. Therefore, health authorities in the western world recommend higher intakes. For example, the US Department of Agriculture / Department of Health and Human Services recommended as part of their food guidance systeme that the daily diet include two to three servings of fruit and three to five servings of vegetables (Dietary Guidelines for Americans, Home and Garden Bulletin No 232. Washington DC: Govt Printing Office, 1980). However, the current trend is an even lower con-

sumption of fruit and vegetables. In the US only 9% of the population actually consumes these recommended servings (Patterson *et al.*, Am. J. Publ. Health, 80, 1443-1449 [1990]).

In the Netherlands the consumption of fruit and vegetables has almost been halved in the last 10 years, irrespective the recommendation to consume 200 g fruits and 200 g vegetables per day. Therefore, to meet the required uptake of antioxidants from fruit and vegetables, antioxidants derived from fruit and vegetables should be added to foods, food supplements or pharmaceutical preparations to overcome this problem. From the recommended daily intake of about 400 to 500 g of fruit and vegetables it can be estimated that about 6 to 10 mg of carotenoids in total is consumed.

Zhou *et al.* studied the relative bioavailability of ß-carotene and α-carotene from different extracts of carrots. From this study it appears that the food matrix and the crystalline form of carotenoids in carrot chromoplasts reduce the bioavailability of carotenoids from carrot juice (Zhou *et al.*, J. Am. Coll. Nutr., 15, 84-91[1996]).

At present published absorption studies have mainly been conducted with β-carotene. In a review article Erdmann *et al.* reported that these studies suggest that absorption of β-carotenoid, a process which is very complex and to a large degree poorly understood, is highly dependent on the amount of fat present in the food (Erdman *et al.*, Ann. N.Y. Acad. Sci., 691, 76-85 [1993]).

The prior art has provided food products comprising added antioxidants. WO 97/06697 discloses a food product comprising natural components which increase the antioxidant status in the blood plasma of the consumer of the food product. This food product comprises antioxidants and/or antioxidant vitamins and preferably a mixture of at least two of these antioxidants and/or antioxidant vitamins, said antioxidants and/or antioxidant vitamins being preferably selected from α-tocopherol (vitamin E), ascorbic acid (vitamin C), polyphenols and carotenoids, in such amounts that upon daily consumption of the daily average amount of the food product the antioxidant status in the human blood plasma is increased significantly. The food product may comprise in particular per amount of the average daily consumed amount of the food product 30-

100 mg of α-tocopherol and/or 120-600 mg of ascorbic acid and/or 2-18 mg of - a mixture of - carotenoids and/or 0.5-2.5 g of - a mixture of - tea polyphenols and/or 25-125 mg of - a mixture of - polyphenols not being derived from tea.

WO 96/39869 also discloses a food product containing a blend of antioxidants and a edible second component selected from carbohydrates, fats and proteins, wherein said antioxidants may be nutritive antioxidants such as provitamin A carotenes, vitamin C and vitamin E or non-nutritive antioxidants such as non-provitamin A carotenes and antiinflammatory agents.

Furthermore, prior art is known which relate to the pharmaceutical use of antioxidants. WO 96/40092 discloses the pharmaceutical application of the carotenoids lutein and zeaxanthin in the treatment or prophylaxis of disease of the macula, more particularly the age-related macular degeneration. WO 96/19215 discloses a method for the prevention or treatment of atherosclerosis in a mammal, wherein an effective amount of natural tocopherol and natural carotene is administered to said mammal.

The prior art therefore discloses mixtures of naturally occurring antioxidants including naturally occurring carotenoids and the use thereof in food products, food supplements and pharmaceutical preparations. However, mixtures of naturally occurring carotenoids have several disadvantages. First, oleoresins containing mixtures of carotenoids are also extracted from natural sources and have a large variation of carotenoid crystals and/or amorphous particles having sizes which vary typically from 5 to 300 μm, whereas the majority of said crystals and/or amorphous particles is in the 200 - 300 μm range. This high variation in particle size leads to inhomogenicity and instability of the carotenoid blend as the particles may easily agglomerate. When used in beverages such as non-fruit juice drinks, fruit juices, carbonated drinks, milk based drinks and sportdrinks, inhomogenic and unstable beverages are obtained. Second, agglomeration and/or recrystallisation of the carotenoid crystals and/or amorphous particles leads to larger carotenoid comprising particles which are more difficult to solubilize into micellar systems in the small intestine lumen and, as a consequence, the absorption of carotenoids is suboptimal.

Hence, the uptake of carotenoids in the intestine is affected when larger particles are formed which means that the bioavailability thereof is reduced. The relevance of the particle size to bioavailability has not been accounted for by the prior art. A further disadvantage of large crystals and/or particles shows up in soft gelatin capsules as they often induce leakage of said capsules.

Summary of the invention

The present invention provides a solution for the problems mentioned hereinabove in particular those related to the large variation of the size of carotenoids and/or amorphous particles. The present invention therefore relates to a preparation of an improved mixture of naturally occurring antioxidants having a physical form and particle size which confers stability thereof in products such as food products and does not disrupt the product structure so that bioavailability of the antioxidants is improved, e.g. the care and/or cure of the physiological antioxidant status of a mammal and of prokaryotic and eucaryotic cells. The present invention relates more in particular to a preparation for the enhancement of the antioxidant status of cells which comprises a mixture of antioxidants, wherein the mixture comprises at least (a) two naturally occurring carotenoids, (b) a naturally occurring tocopherol or a derivative thereof, and (c) vitamin C, wherein the naturally occurring carotenoids are in the form of carotenoid containing particles having an average diameter of 0.01 to 100 μm.

Detailed description of the invention

The present invention relates to a preparation comprising a mixture of antioxidants, wherein the mixture comprises at least (a) two naturally occurring carotenoids, (b) a naturally occurring tocopherol or a derivative thereof, and (c) vitamin C, wherein the naturally occurring carotenoids are in the form of carotenoid containing particles having an average diameter of 0.01 to 100 μm.

As noted herein above Erdmann *et al.* reported that absorption of β-carotene, is a process which appears to be highly dependent from the amount of fat present in the food. However, comparing the absorption of synthetic all-*trans* β-carotene vs. naturally occurring

carotenoids, *i.e.* α- and β-carotenes and other carotenoids extracted from palm fruits, lutein and zeaxanthin from marigold flowers, it was found that the absorption of carotenoids in rats of specific natural carotenoids consisting of mixtures of *cis* and *trans* isomers extracted from palm fruit and marigold flowers was not effected by the amount of fat in the food, whereas the amount of absorbed synthetic all-*trans* β-carotene strongly correlated with the amount of fat in the diet. This implies that low, very low fat and non fat containing food products as well as the well known high fat food products such as margarine and spreads are good vehicles for the absorption of supplemented carotenoids.

However, also when enough fat is available in the food, the size of the carotenoid particles influences the bioavailability of the carotenoids. The smaller the particles the better the bioavailability of the carotenoids. Therefore, also in a high fat food product the size and the nature of the carotenoid particle determines the level of its bioavailability.

Another limitation of the absorption of synthetic all-*trans* β-carotene from low or non fat preparations is the crystallisation or agglomeration of all-*trans* β-carotene to large crystals or large agglomerates in aqueous media such as beverages and other emulsion systems. In practice this means that these large particles will not fully solubilize into micellar systems in the intestinal lumen. Therefore, the absorption of these carotenoids in the intestine is suboptimal. The application of the preparation according to the invention in aqueous media, however, results into highly stable media from which these carotenoids can effectively be absorbed in high amounts.

By comparing prior art data relating to the blood levels of carotenoids associated with the consumption of different types of vegetables with data obtained from human trials with supplemented foods comprising the preparation according to the invention the carotenoids present in the supplemented foods, which were part of a normal diet, were absorbed about 4 to 10 times more efficiently than those present in the vegetables in the diet. Hence, the amount of carotenoids for supplementing a diet can be 4 to 10 times lower than the amount which is only based on the carotenoid content of

vegetables. For example, Martini *et al.* (Cancer Epidemiol. Biomarkers & Prev., 4, 491-496 [1995]) shows in a 9 days human feeding study that an experimental high vegetable carrots-spinach diet 1 mg α-carotene in the diet gives an α-carotene rise of 0.0371 µmol/L in the blood plasma whereas 1 mg β-carotene rises the plasma concentration of β-carotene with 0.0137 µmol/L. In a human trial with a daily intake of 15 g margarine containing palm fruit carotenes (3 mg α-carotene and 9 mg β-carotene, carotenoid particle size 10 µm) it was found that 1 mg α-carotene by margarine rises the bloodplasma concentration with 0.2200 µmol/L whereas 1 mg β-carotene by margarine rises the bloodplasma concentration with 0.0867 µmol/L. Comparing the changes in carotenoid bloodplasma levels per mg of the individual carotenoid consumed, it can be seen that the uptake from margarine is about 6 times more efficient than from the carrot-spinach diet. Although a direct comparison is difficult to make because of differences in duration of the study, the data suggest that the amounts of carotenoids to supplement the diet by a food or food supplement can be several times lower than amounts simply based on the carotenoid content of vegetables. That means that consumption of only a little amount of the preparation according to the invention can be regarded as equivalent to the consumption of - much higher - amounts of carotenoids as present in fruits and vegetables and that for the prevention of degenerative diseases in normal situations only the consumption of a small amount of the preparation according to the invention is sufficient. In case of enhanced oxidative stress or diseases, the consumed amount of the preparation according to the invention is assumed to be several times higher than under normal situations.

Additionally, leakage of soft gelatin capsules comprising naturally occurring carotenoids was found to be - at least in part - due to relatively large carotenoid crystals and/or agglomerates which are formed in time as a result of recrystallization or agglomeration of carotenoids. According to the invention this problem is overcome by controlling the size of the carotenoid crystals and/or agglomerates. According to the invention the preparation contains at least two naturally occurring carotenoids which are obtained by milling carotenoid extracts in a milling

machine such as a ball-milling machine, so that the naturally occurring carotenoids are in the form of carotenoid containing particles having an average diameter of 0.01 to 100 µm are obtained. Preferably the milling is carried out in such a way that the average diameter of the carotenoid containing particles is in the range of 0.1 to 50 µm.

According to the invention it is furthermore preferred that the preparation containing carotenoid particles having an average diameter of 0.01 to 100 µm are prepared by blending oleoresins comprising carotenoids and at least the other components (b) and (c) followed by milling in e.g. a ball-milling machine.

By the milling step the agglomerated carotenoids and the large carotenoid crystals are reduced in size. Preferably reagglomeration of the carotenoid particles having an average diameter of 0.01 to 100 µm is prevented by adding a one or more surface active agents. According to the invention a suitable and preferred surface active agent is lecithin.

Typically the carotenoid particles have an average diameter of about 10 µm within the range of 0.1 to 50 µm as analyzed by laser size recording. On a routine base the particles can be qualitatively sized by microscopic examination.

A stable mixture of carotenoid containing particles as contained by the preparation according to the invention can also be achieved by encapsulating. The process of encapsulation according to the invention involves the following steps: solubilisation and milling, e.g. ball-milling, of carotenoids, dispersion of this solution into an aqueous emulsion base - particle size reduction / further emulsification by high pressure homogenisation. According to the invention various additional functional antioxidants, e.g. ascorbic acid, ascorbic palmitate and tocopherols may be added to enhance the product shelf life. Additionally, emulsifiers may be added which encapsule the small droplets of carotenoids and forming the phase interface. Other components such as carbohydrates may be used to increase the protection of the encapsulate. According the invention the emulsifiers used are preferably modified starch, gelatin from various sources such as fish gelatin, gum arabic and milk proteins. In this way encapsulated blends of carotenoids and/or

curcumin can be obtained wherein the particles have an average diameter of 0.1 to 10 μm.

The invention further provides water soluble emulsions of carotenoids which are made by high pressure homogenisation. In these solutions the particle size is on average in the range of 0.5 - 2.0 μm. The emulsified mixtures or blends of carotenoids as obtained by encapsulation may optionally contain curcumin.

The preparation according to the invention can be used in soft gelatine capsules which are highly stable and non leaking capsules. The preparation according to the invention may also be used in fat, low or non fat foods in which the preparation is homogenously and stably distributed. According to the invention the uptake of the carotenoids in the highly bioavailable form as disclosed herein combined with the components (b) and (c) leads to an enhanced antioxidant management in the cells and tissues and, as a consequence, to less oxidative damage.

Because of the efficacy of the preparation according to the invention, the levels at which the preparation is applied in various products can be relatively low in comparison with levels of the normally used antioxidant mixtures derived from fruit and vegetables.

The component (a) of the preparation according to the invention is preferably selected from the group of carotenoids comprising a α-carotene, a β-carotene, a γ-carotene, lycopene, lutein, zeaxanthin, capsanthin, capsorubin or a mixture thereof, wherein these carotenoids may be in the *cis* and/or *trans* configuration. According to the invention, however, these carotenoids are a mixture of *cis* and *trans* isomers.

The component (b) of the preparation according to the invention is preferably selected from α-, β-, γ-, δ-tocopherol, a mixture of these tocopherols, α-, β-, γ-, δ-tocotrienol or a mixture of tocotrienols or a mixture of these tocopherols and tocotrienols.

The preparation according to the invention preferably comprises also one or more of the following antioxidants:

- one or more antioxidants selected from the group of polyphenols derived from fruits, preferably red fruits and in particular grapes, wherein the polyphenols may be flavonoids.

The antioxidants intended here may also be derived from vegetables and products derived thereof such as tea, wine, beer or other fermented beverages and/or products derived thereof.

- a vitamin B, in particular folate, vitamin B_6, vitamin B_{12} or a mixture thereof.
- an antioxidant selected from spices, herbs, antioxidants derived from natural extracts of fruits and vegetables, or a mixture of at least of two of these antioxidants. A preferred spice is curcumin. A preferred herb is rosemary or tea or a mixture thereof.
- an antioxidant mineral. According to the invention the antioxidant mineral is selected from selenium, manganese, iron, copper, zinc or a mixture thereof.
- an antioxidant selected from antioxidant peptides, protein hydrolysates containing small peptides, antioxidant proteins or enzymes, or a mixture thereof. According to the invention the antioxidant peptide is preferably gluthatione. The antioxidant protein is preferably catalase, superoxide dismutase, a peroxidase, lactoferrin, transferrin, ferritin, caeruloplasmin, albumin or a mixture thereof, whereas the peroxidase is preferably gluthatione peroxidase, horseradish peroxidase, lactoperoxidase or a mixture thereof.
- an additional antioxidant selected from uric acid, taurine, cysteine, Coenzym Q, bilirubin, or a mixture thereof. More preferably the preparation comprises as the additional antioxidant uric acid or taurine or a mixture thereof.

The preparation according to the invention may be used in a food product or food supplement which preferably comprises an amount of 0.01 to 10 mg of a mixture of at least two naturally occurring carotenoids, said amount being based on the average daily doses of the preparation. According to the invention the food product or food supplement is contained by a soft gelatine capsule.

The preparation according to the invention may be used in a pharmaceutical composition, wherein the preparation preferably contains an amount of 0.01 to 5 mg ß-carotene, said amount being based on the average daily doses of the pharmaceutical composition.

The preparation according to the invention may further be used in a cell, tissue, culture or fermentation medium.

Examples

Example 1

Food supplement for the prevention of degenerative diseases in general (1 or 2 capsules/day).

Soft gelatin capsule containing the following antioxidant mix:

- 100 mg Vegex® oil soluble carotenoid mix with particle size of 1 to 30 µm:
  Blend oil soluble carotenoid mix of 100 mg of Vegex® mix composed out of 9.4 mg naturally occurring carotenoids, blend and ball milled mix composed out of 2 parts Vegex® palm carotene OS30, 1 part Vegex® lycopene OS12 and 1.7 part Vegex® lutein OS20. The carotenoid composition per capsule is 1.9 mg α-carotene, 3.4 mg β-carotene and 0.1 mg γ-carotene, 1.9 mg lutein and 0.1 mg zeaxanthin, 2.0 mg lycopene,
- 200 mg Vegex® red wine grape polyphenols: 80 mg polyphenols derived from grape skins and grape seeds,
- 10 mg vitamin E,
- 60 mg vitamin C (ascorbic acid),
- 4 mg vitamin B-complex, including 2 mg vitamin B_6, 1 µg vitamin B_{12}, 100 µg folic acid.

Slow release leakage of soft gelatine capsules during storage at room temperature. 10.000 soft gelatin capsules were taken from a batch of 50.000 capsules and divided over 100 bottles of glass containing 100 capsules each. The leakage is shown as a yellow/orange liquid on the bottom of the bottle. The compositions of the capsules are the same as example 1 with respect to overall composition, however the particle size are different: the formula according to the state of the art has an average particle diameter of 2 - 300 µm, whereas the formula according to the invention has an average homogeneous particle diameter of 1 to 30 µm.

	Formula (state of the art) Particle size: 2 - 300 µm	Formula (invention) Particle size: 1 - 30 µm
Week	Leakage % cumulative	Leakage % cumulative
0	0	0
2	2	0
4	5	0
6	8	0
8	11	0
10	18	0
12	25	1
14	43	1
16	70	1

Vegex®, a registered trademark of Quest International, is a range of vegetable extracts such as mixes of carotenoids, curcumin, polyphenols etc.

Example 2

Food supplement for protection against cardiovascular disease in high risk groups (2 capsules/day):

Hard gelatin capsule containing the following antioxidant mix:

- 200 mg Vegex® encapsulated carotenoid blend and curcumin mix with particle size of 0.1 to 10 µm containing 1.9 mg α-carotene, 3.4 mg β-carotene and 0.1 mg γ-carotene, 1.9 mg lutein and 0.1 mg zeaxanthin, 2.0 mg lycopene, and 10 mg curcumin,
- 150 mg Vegex® red wine grape polyphenols: 60 mg polyphenols derived from grape skins and grape seeds,
- 20 mg vitamin E,
- 100 mg vitamin C: ascorbic acid (encapsulated) or ascorbyl palmitate,
- 4 mg vitamin B-complex, including 2 mg vitamin B_6, 1 µg vitamin B_{12}, 100 µg folic acid.

Example 3

Food supplement for the prevention of degenerative diseases in general (1 or 2 tablets/day).

Direct compression tablet of 500 mg containing the following antioxidant mix:

- 100 mg Vegex® encapsulated carotenoid mix with particle size of 0.3 to 3 µm, per tablet is 1.9 mg α-carotene, 3.4 mg β-carotene and 0.1 mg γ-carotene, 1.9 mg lutein and 0.1 mg zeaxanthin, 2.0 mg lycopene,
- 100 mg Vegex® encapsulated curcumin : 10 mg curcumin,
- 100 mg Vegex® red wine grape polyphenols: 40 mg polyphenols derived from grape skins and grape seeds,
- 10 mg vitamin E,
- 60 mg vitamin C: ascorbic acid (encapsulated),
- 4 mg vitamin B-complex, including 2 mg vitamin B_6, 1 µg vitamin B_{12}, 100 µg folic acid.

Example 4

Enteral (clinical) feedings of 1 litre containing the following antioxidant mix:

- 1 ml blend of water dispersible carotenoids with particle size 1 - 7 µm containing a mix of 6.5 g of naturally occurring carotenoids. The 1000 ml blend of water dispersible carotenoids is composed from 133 ml Vegex® palm carotene WS2, 700 ml Vegex® lutein WS1, 100 ml Vegex® lycopene WSR and 66 ml Vegex® capsicum WS1. The carotenoid composition of 1000 ml carotenoid blend is 122 mg α-carotene, 275 mg β-carotene and 2 mg γ-carotene, 299 mg lutein and 32 mg zeaxanthin, 76 mg lycopene, 102 mg capsanthin + capsorubin,
- 20 mg vitamin E (α-tocopherol),
- 100 mg vitamin C: ascorbic acid.

Example 5

A soft drink beverage for protection in an air polluted environment containing per litre the following antioxidant mix:

- water dispersible Vegex® WS carotenoid blend with particle size of 0.5 to 2.0 µm containing a mix of 6 mg of naturally

occurring carotenoids: 1.2 mg α-carotene, 2.2 mg β-carotene and 0.06 mg γ-carotene, 1.2 mg lutein and 0.06 mg zeaxanthin, 1.3 mg lycopene,

- 20 mg vitamin E,
- 120 mg vitamin C: ascorbic acid or sodium ascorbate,
- a mineral such as a salt containing 30 mg Zn,
- 2 g taurine.

Example 6

Vegetable-fruit bar containing the following antioxidant mix:

- blend of oil soluble carotenoids containing a mix of 0.01 to 10 mg of naturally occuring carotenoids per daily dosis (1 - 2 bars of 34 g each): 10 mg Vegex® palm carotene OS30 with particle size typically 10 µm: 1.0 mg α-carotene, 1.8 mg β- and 0.05 mg γ-carotene,
- 6 mg Vegex® lutein OS30 with particle size 5 - 50 µm: 0.2 mg lutein and 0.01 mg zeaxanthin,
- 12 mg Vegex® lycopene WSR with particle size 0.5 - 2.0 µm: 0.06 mg lycopene,
- 75 mg Vegex® red wine grape polyphenols: 30 mg polyphenols derived from grape skins and/or grape seeds,
- 5 mg vitamin E,
- 30 mg vitamin C.

Example 7

Bread containing the following antioxidant mix per 100 g of bread (3 slices of bread):

- blend of oil soluble Vegex® lutein OS30 with particle size 5 - 50 µm: 0.2 mg lutein plus 0.01 mg zeaxanthin,
- 2 mg vitamin E: α-tocopherol (as found in Biopherol® 50),
- 12 mg vitamin C: encapsulated ascorbic acid.

Biopherol® is a registered trademark of Quest International containing mixtures of natural tocopherols.

Example 8

A cell culture medium for growth and maintenance of anti CD20, a hybridoma cell line. The cell culture medium was composed on basis of the well known RPMI 1640 medium. This medium was prepared from the RPMI-1640 select kit from Gibco BRL, Life Technologies Inc, Cat No. 17402-017. The cel culture of media medium contains among others free amino acids, glucose, certain minerals, riboflavin, and B vitamins.

The following antioxidant mix is added to 1 liter of the medium:

- 0.01 ml Vegex® oil soluble carotenoid mix with particle size of 3 to 30 µm emulgated with e.g. Pluronic F-68 and Tween 80 containing 19 µg α-carotene, 34 µg β-carotene and 1 µg γ-catotene, 19 µg lutein and 1µg zeaxanthin and 20 µg lycopene,
- 10 mg Vegex® red wine grape polyphenols: 4 mg polyphenols derived from grape skins and/or grape seeds,
- 0.5 g vitamin E as α-tocopherol emulgated with e.g. Pluronic F-68 (Life Technologies Inc) and Tween 80,
- 0.2 g vitamin C: ascorbic acid or sodium ascorbate,
- antioxidants such as uric acid (10 mg), bilirubin (6 mg), taurine (0.1 g), albumin (40 mg), antioxidant peptides, e.g. gluthatione (1 mg) and specific protein hydrolysates (0.1 g) containing small peptides with e.g. aromatic amino acids,
- 2 mg zinc.

Claims

1. Preparation comprising a mixture of antioxidants, wherein the mixture comprises at least:

(a) a mixture of at least two naturally occuring carotenoids

(b) a naturally occuring tocopherol or a derivative thereof, and

(c) vitamin C,

wherein the naturally occuring carotenoids are in the form of carotenoid containingparticles having an average diameter of 0.01 to 100 µm.

2. Preparation according to claim 1, wherein the naturally occuring carotenoid is α-carotene, β-carotene, γ-carotene, lycopene, lutein, zeaxanthin, capsanthin, capsorubin or a mixture thereof.

3. Preparation according to claim 1 or claim 2, wherein the naturally occuring tocopherol or the derivative thereof is α-tocopherol, a mixture of tocopherols, a tocotrienol, a mixture of tocotrienoles or a mixture thereof.

4. Preparation according to any one of claims 1-3, wherein the mixture of antioxidants comprises at least a polyphenol which is derived from fruits.

5. Preparation according to claim 4, wherein the fruits are selected from grapes.

6. Preparation according to any one of claims 1-5, wherein the mixture of antioxidants comprises at least a vitamin B.

7. Preparation according to claim 6, wherein the vitamin B is folate, vitamine B_6, vitamine B_{12} or a mixture thereof.

8. Preparation according to any one of claims 1-7, wherein the mixture of antioxidants comprises at least an antioxidant selected from spices, herbs, antioxidants derived from natural extracts of fruits and vegetables, or a mixture of at least of two of these antioxidants.

9. Preparation according to claim 8, wherein the spice is curcumin.

10. Preparation according to claim 8, wherein the herb is rosemary or tea or a mixture thereof.

11. Preparation according to any one of claims 1-10, wherein the mixture of antioxidants comprises at least an antioxidant mineral.

12. Preparation according to claim 11, wherein the antioxidant mineral is selenium, manganese, iron, copper, zinc or a mixture thereof.

13. Preparation according to any one of claims 1-12, wherein the mixture of antioxidants comprises at least an antioxidant selected from antioxidant peptides, protein hydrolysates containing small peptides, antioxidant proteins or enzyms, or a mixture thereof.

14. Preparation according to claim 13, wherein the antioxidant peptide is gluthatione.

15. Preparation according to claim 13, wherein the antioxidant protein is catalase, superoxide dismutase, a peroxidase, lactoferrin, transferrin, ferritin, caeruloplasmin, albumin or a mixture thereof.

16. Preparation according to claim 15, wherein the peroxidase is gluthatione peroxidase, horseradish peroxidase, lactoperoxidase or a mixture thereof.

17. Preparation according to any one of claims 1-16, wherein the mixture of antioxidants comprises an additional antioxidant selected from uric acid, taurine, cysteine, Coenzym Q, bilirubin, or a mixture thereof.

18. Preparation according to claim 17, wherein the additional antioxidant is uric acid or taurine or a mixture thereof.

19. Food product or food supplement comprising the preparation according to any one of claims 1-18.

20. Food product ot food supplement according to claim 19, wherein the food product comprises an amount of 0.01 to 10 mg of naturally occuring carotenoides, said amount being based on the average daily dosis of the preparation.

21. Food product or food supplement according to claim 19 or claim 20, wherein the food product or the food supplement is contained by a soft gelatine capsule.

22. Pharmaceutical composition comprising the preparation according to any one of claims 1-18.

23. Cell, tissue, culture or fermentation medium comprising the preparation according to any one of claims 1-18.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/NL 97/00662

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A23L1/302 A23L1/304 A23L1/305 A61K45/06

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A23L A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
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Y,X	DATABASE WPI Section Ch, Week 9620 Derwent Publications Ltd., London, GB; Class B05, AN 96-196556 XP002072436 & JP 08 067 666 A (LION CORP) see abstract	1-3, 8-10, 19-23
Y,X	PATENT ABSTRACTS OF JAPAN vol. 096, no. 007, 31 July 1996 -& JP 08 067666 A (LION CORP), 12 March 1996, see abstract	1-3, 8-10, 19-23

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

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Date of the actual completion of the international search	Date of mailing of the international search report
23 July 1998	04/08/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Van Moer, A

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 94 22322 A (KALAMAZOO HOLDINGS INC) 13 October 1994 see page 9, line 30; claims 1,3,5-10	1-10, 19-23
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Inter...onal Application No
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